

WE CLAIM:

1. A method for inactivating a nuclear localization signal of a protein comprising contacting the protein with a compound that is capable of stable reversible binding with 5 basic amino acid residues of the nuclear localization signal of the protein.

2. A method for inhibiting importation of a protein into the nucleus of a cell comprising contacting the protein 10 with a compound that is capable of stable reversible binding with basic amino acid residues of the nuclear localization signal of the protein.

3. A method for targeted inactivation of a nuclear 15 localization signal of a protein in a complex comprising contacting the protein with a compound that is capable of:

(a) interacting with a molecule in a complex having a specific docking site which is positioned proximately to a nuclear localization signal of a protein in the 20 complex; and

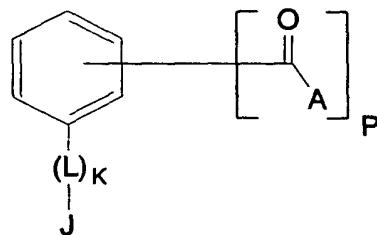
(b) forming stable reversible covalent interactions with basic amino acid residues of the nuclear localization signal of the protein.

25 4. The method of claim 3 wherein the compound forms Schiff bases with lysine residues of the nuclear localization signal of the protein.

5. The method of claim 3 wherein the compound forms 30 stable reversible covalent interactions with arginine residues of the nuclear localization signal of the protein.

6. A method for targeted inactivation of a nuclear localization signal of a protein comprising contacting the 35 protein with a compound according to the formula:

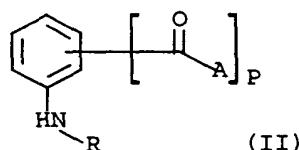
5



(I)

wherein A, independently, = CH_3 , CH_2CH_3 , COH , $COCH_3$, $COCH_2CH_3$, CH_2COCH_3 , $CH_2COCH_2CH_3$, $C(CH_3)_2COCH_3$, or $C(CH_3)_2COCH_2CH_3$; P = 1 or 2; L is a linker group containing an S, O, N or C atom; K = 0 or 1; and wherein J represents (i) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic hydrocarbon group; (ii) a saturated or saturated, substituted or unsubstituted, straight or branched acyclic group containing hetero atoms such as nitrogen, 15 sulfur or oxygen; (iii) a substituted or unsubstituted, saturated or aromatic, mono- or poly- cyclic group having 3 to 20 carbon atoms; or (iv) a substituted or unsubstituted, saturated or aromatic, mono- or poly- heterocyclic group having 3 to 20 atoms, at least one of which is a nitrogen, 20 sulfur or oxygen.

25

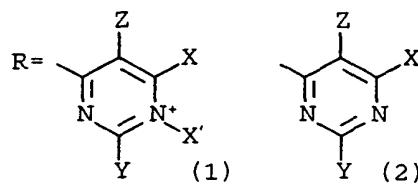


(II)

30

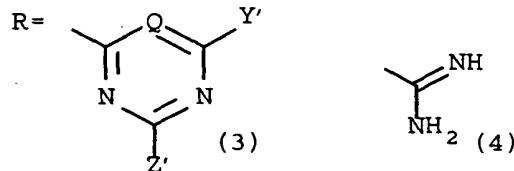
wherein A, independently, = CH_3 , CH_2CH_3 , COH , $COCH_3$, $COCH_2CH_3$, CH_2COCH_3 , $CH_2COCH_2CH_3$, $C(CH_3)_2COCH_3$, or $C(CH_3)_2COCH_2CH_3$; and P = 1 or 2; and

5

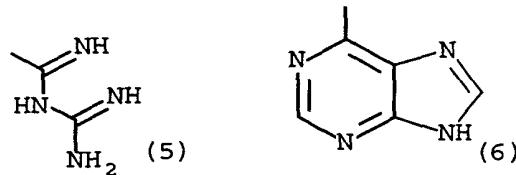


wherein $X = \text{NH}_2, \text{CH}_3$ or CH_2CH_3 ; $X' = \text{CH}_3$ or CH_2CH_3 ; $Y = \text{NH}_2, \text{NHCH}_3, \text{N}(\text{CH}_3)_2$, 1-pyrrolidino or 1-piperidino; and $Z = \text{H}, \text{CH}_3$ or CH_2CH_3 ; or

15



20

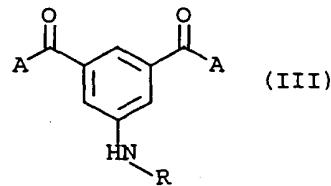


wherein Y' and Z' , independently, = $\text{H}, \text{NH}_2, \text{NHCH}_3, \text{N}(\text{CH}_3)_2, \text{N}^+(\text{CH}_3)_3$, 1-pyrrolidino or 1-piperidino; Q is N or CH ; and salts thereof.

25

8. A method for targeted inactivation of a nuclear localization signal of a protein comprising contacting the protein with a compound according to the formula:

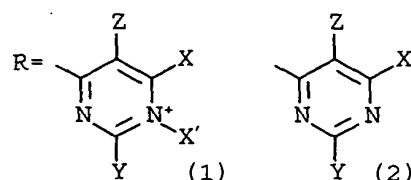
30



35

wherein A = CH_3 , CH_2CH_3 , COCH_3 , COCH_2CH_3 , CH_2COCH_3 , $\text{CH}_2\text{COCH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2\text{COCH}_3$ or $\text{C}(\text{CH}_3)_2\text{COCH}_2\text{CH}_3$; and

5

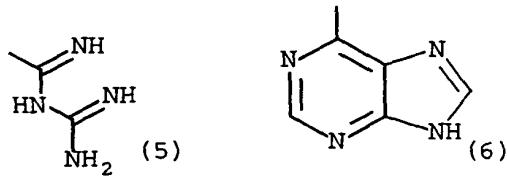
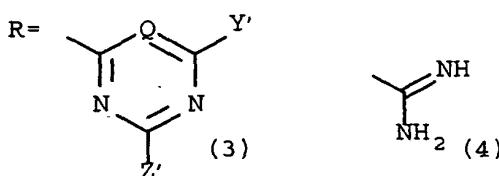


10

wherein X = NH₂, CH₃ or CH₂CH₃; X' = CH₃ or CH₂CH₃; Y = NH₂, NHCH₃, N(CH₃)₂, 1-pyrrolidino or 1-piperidino; and Z = H, CH₃ or CH₂CH₃; or

15

20



wherein Y' and Z', independently, = H, NH₂, NHCH₃, N(CH₃)₂,
30 N⁺(CH₃)₃, 1-pyrrolidino or 1-piperidino; Q is N or CH; and
salts thereof.

9. The method of claim 3, 4 or 5 wherein the docking site is on the protein having the nuclear localization signal.

10. The method of claim 3, 4, 5, 6, 7 or 8 wherein the protein is derived from a human immunodeficiency virus, influenza virus, hepatitis virus, herpes simplex virus, papillomavirus, parvovirus or measles virus.

5

11. The method of claim 3 wherein the docking site is on the human immunodeficiency virus reverse transcriptase and the nuclear localization signal is in the human immunodeficiency virus matrix antigen.

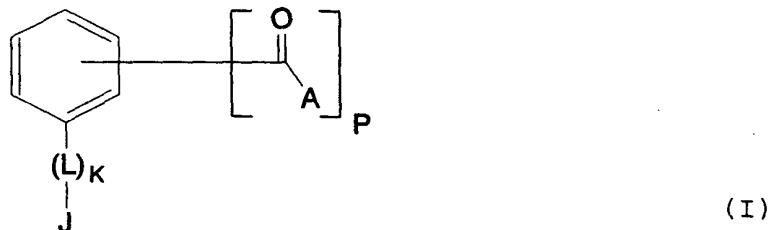
10

12. A method for identifying compounds that are capable of targeted inactivation of the nuclear localization signal of a protein comprising:

15

(a) contacting an immobilized cellular receptor moiety with a protein comprising a nuclear localization signal, and a compound having the formula :

20



25

wherein A, independently, = CH_3 , CH_2CH_3 , COH , COCH_3 , COCH_2CH_3 , CH_2COCH_3 , $\text{CH}_2\text{COCH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2\text{COCH}_3$, or $\text{C}(\text{CH}_3)_2\text{COCH}_2\text{CH}_3$; P = 1 or 2; L is a linker group containing an S, O, N or C atom; K = 0 or 1; and wherein J represents (i) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic hydrocarbon group; (ii) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic group containing hetero atoms such as nitrogen, sulfur or oxygen; (iii) a substituted or unsubstituted, saturated or aromatic, mono- or poly- cyclic group having 3 to 20 carbon atoms; or (iv) a substituted or unsubstituted, saturated or aromatic, mono- or poly- heterocyclic group having 3 to 20 atoms, at least one of which is a nitrogen, sulfur or oxygen;

30

35

(b) measuring the binding of the protein to the immobilized cellular receptor moiety; and
(c) comparing the quantity of the protein bound to the quantity of protein bound in the absence of the
5 compound,

where a reduction in the quantity of the bound protein in the presence of the compound is indicative of targeted inactivation of the nuclear localization signal by the compound.

10

13. The method of Claim 12 wherein the protein is in a complex.

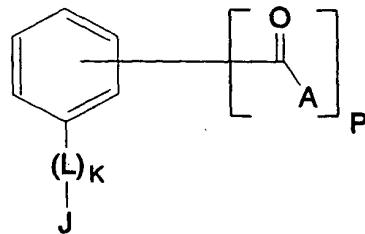
14. The method of Claim 12 wherein the protein is
15 derived from a human immunodeficiency virus, influenza virus, hepatitis virus, herpes simplex virus, papillomavirus, parvovirus or measles virus.

15. The method of Claim 12 wherein the cellular
20 receptor moiety is karyopherin α .

16. A method for identifying compounds that are capable of targeted inactivation of the nuclear localization signal of a viral nucleoprotein complex comprising:

25 (a) contacting an immobilized karyopherin α with a viral nucleoprotein complex contained in a cytoplasmic extract, said complex comprising viral nucleic acid and said cytoplasmic extract prepared from cells infected by the virus, and a compound having the formula :

30



(I)

35

wherein A, independently, = CH_3 , CH_2CH_3 , COH , COCH_3 , COCH_2CH_3 , CH_2COCH_3 , $\text{CH}_2\text{COCH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2\text{COCH}_3$, or

$C(CH_3)_2COCH_2CH_3$; $P = 1$ or 2 ; L is a linker group containing an S , O , N or C atom; $K = 0$ or 1 ; and wherein J represents (i) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic hydrocarbon group; (ii) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic group containing hetero atoms such as nitrogen, sulfur or oxygen; (iii) a substituted or unsubstituted, saturated or aromatic, mono- or poly- cyclic group having 3 to 20 carbon atoms; or (iv) a substituted or unsubstituted, saturated or aromatic, mono- or poly- heterocyclic group having 3 to 20 atoms, at least one of which is a nitrogen, sulfur or oxygen;

5 (b) measuring the binding of said complex to the immobilized karyopherin α by quantitating the amount of viral nucleic acids associated with said complex; and

10 (c) comparing the quantity of the nucleic acid bound to the quantity of nucleic acid bound in the absence of the compound;

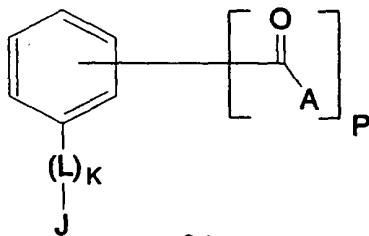
15 20 where a reduction in the quantity of the bound nucleic acid in the presence of the compound is indicative of targeted inactivation of the nuclear localization signal by the compound.

25 17. A compound that is capable of:

(a) interacting with a molecule in a complex having a specific docking site which is positioned proximately to a nuclear localization signal of a protein in the complex; and

30 (b) forming stable reversible covalent interactions with basic amino acid residues of the nuclear localization signal of the protein; and having the formula:

35



(I)

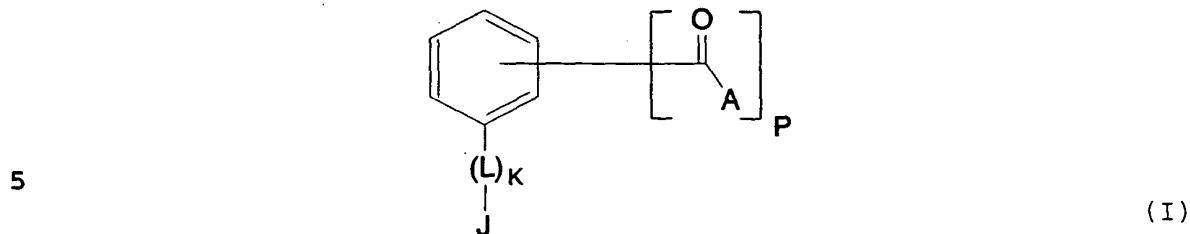
wherein A, independently, = CH_3 , CH_2CH_3 , COH , COCH_3 ,
5 COCH_2CH_3 , CH_2COCH_3 , $\text{CH}_2\text{COCH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2\text{COCH}_3$, or
 $\text{C}(\text{CH}_3)_2\text{COCH}_2\text{CH}_3$; P = 1 or 2; L is a linker group
10 containing an S, O, N or C atom; K = 0 or 1; and wherein
J represents (i) a saturated or unsaturated, substituted
or unsubstituted, straight or branched acyclic
hydrocarbon group; (ii) a saturated or unsaturated,
substituted or unsubstituted, straight or branched
acyclic group containing hetero atoms such as nitrogen,
15 sulfur or oxygen; (iii) a substituted or unsubstituted,
saturated or aromatic, mono- or poly- cyclic group
having 3 to 20 carbon atoms; or (iv) a substituted or
unsubstituted, saturated or aromatic, mono- or poly-
heterocyclic group having 3 to 20 atoms, at least one of
15 which is a nitrogen, sulfur or oxygen.

18. The compound of Claim 17 wherein the protein is
derived from a virus.

20 19. The compound of Claim 17 wherein the protein is
derived from a human immunodeficiency virus, influenza virus,
hepatitis virus, herpes simplex virus, papillomavirus,
parvovirus or measles virus.

25 20. A method of preventing productive infection by a
virus of a proliferating population of cells, which comprises
preventing importation of a complex containing viral nucleic
acid or viral protein into the nucleus of a cell in the
30 population.

35 21. The method of claim 20 which further comprises the
administration of an effective amount of a pharmaceutical
composition containing a compound according to the formula:



wherein A, independently, = CH_3 , CH_2CH_3 , COH , COCH_3 , COCH_2CH_3 , CH_2COCH_3 , $\text{CH}_2\text{COCH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2\text{COCH}_3$, or $\text{C}(\text{CH}_3)_2\text{COCH}_2\text{CH}_3$; P = 1 or 2; L is a linker group containing an S, O, N or C atom; K = 0 or 1; and wherein J represents (i) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic hydrocarbon group; (ii) a saturated or unsaturated, substituted or unsubstituted, straight or branched acyclic group containing hetero atoms such as 10 nitrogen, sulfur or oxygen; (iii) a substituted or unsubstituted, saturated or aromatic, mono- or poly- cyclic group having 3 to 20 carbon atoms; or (iv) a substituted or unsubstituted, saturated or aromatic, mono- or poly- 15 heterocyclic group having 3 to 20 atoms, at least one of which is a nitrogen, sulfur or oxygen.

20

22. The method of claim 20 which comprises the administration of an effective amount of a pharmaceutical composition containing Compound No. 2 as an active 25 ingredient.

23. The method of claim 1 wherein the compound is capable of forming tandem Schiff bases with lysine residues of the nuclear localization signal of the protein.

30

24. The method of claim 1 wherein the compound is capable of forming stable reversible adducts with arginine residues of the nuclear localization signal of the protein.

35 25. The method of claim 3, 4, 5, 6, 7 or 8 wherein the protein is a transcription factor.